Biolog ECOplate extractions for bacterial diversity and abundance
Modified from Treseder lab protocol

***All pipette tips, utensils, solutions, and containers must be sterile (autoclaved)**
Perform procedure in hood and wear gloves

Autoclaving pipette tips: wrapped dry load, 20 minutes at 121C, exhaust 40 minutes; use autoclave tape on tip boxes to make sure that sterilization occurs.

For water-extracted samples (leachate samples, etc):
Materials

Sterile sample cups (1 per sample)
Orange pipette tips (5-300µL) and pipette (multichannel preferred)
Biolog ECOplates (one per 3 samples)
Disposable pipetting reservoir (sterilize in bleach water and rinse well; do not autoclave)

1) Collect leachate in sterile sample cups. It is best to inoculate the plates the same day as sample collection. Remember to collect water to use as a blank.
2) Inoculate 150µL of sample directly into each of the 32 wells designated as a sample section on the biolog plate. If using a multichannel pipette, empty the sample cup into the pipetting reservoir.
3) Store at room temperature (or 20C incubator) until ready to read on microplate reader. Read at 590nm on microplate reader ("biolog" protocol on KC4 software). For Arb leachate samples, take readings at approx. 36, 48, 60, and 72 hours after inoculation. To minimize contamination, keep the lid on the plate until right before reading and replace it immediately after.

For unextracted soil samples:
Materials

0.87% saline solution (8.7 g NaCl/L w/v)
Sample cups (1 per sample)
Culture tubes with caps (4 per sample)
1-5 mL pipette with tips
200 µL pipette with tips
Biolog ECOplates (1 per 3 samples)
Vortex mixer (if available)

1) For each sample, set up 4 culture tubes with 9mL saline solution. Label tubes with sample ID and either 1:10, 1:100, 1:1000, or 1:10000
2) Weigh 1.0g soil into sample cup
3) Add 10mL saline to each sample cup and mix gently by hand
4) Add 1mL soil solution to tube labeled "1:10" and vortex; if there is no vortex mix (swirl) vigorously by hand
5) Continue serial dilutions for 1:100, 1:1000, and 1:10000 vials. Remember to mix thoroughly after each dilution.

6) From the 1:10000 tube, inoculate 150µL into each sample section of the biolog plate. Remember one section needs to be a blank (saline solution only).
7) Store at room temperature (or 20C incubator) until ready to read on microplate reader. Read at 590nm ("biolog" protocol on KC4 software). To minimize contamination, keep the lid on the plate until right before reading and replace it immediately after.

Suding Lab
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